33 parts

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INTERFACE PATCH CLAMPING

Introduction

The present invention provides a novel development of the
conventional patch clamp technique. This novel technique
5   is referred to as the interface patch clamp method.

Voltage gated ion channels are potential targets for a
considerable range of novel treatments in a variety of
disease states. The development of the patch clamp
technique has provided a powerful method for the study of
10   ion channel function and pharmacology in whole cells.
However, while the patch clamp technique provides a
definitive method for the investigation and screening of
drugs with potential activity on voltage gated ion
channels, the technique is currently highly dependent on
15   the skill of the operator and tends to be very slow for
drug screening. The present invention provides a method
for increasing the rate at which compounds may be screened
for ion channel blocking/agonist activity using the patch
clamp technique. The method can retain the essential
20   features of the conventional patch clamp recording system
while facilitating automation of the major time-consuming
components of the technique.

Background: Conventional Patch Clamp

The success of the patch clamp technique is derived from
25   the ability to form "tight" (i.e. high resistance: Giga
Ohm) electrical seals between an area of the cell membrane
(the Patch) and the tip of a pipette. The patch clamp
pipette is usually made from glass. The formation of the
G-seal is dependent on the profile of the top of the
30   pipette, and is enhanced by the application of suction to

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the interior of the pipette. The requirements for the
formation of the G-seals are well established and the
process is usually monitored electrically by display of
the current pulse recorded in response to a small voltage
5   step applied throughout seal formation. After formation
of a G-seal, the area of membrane under the pipette may be
disrupted to obtain whole cell voltage clamp recording
mode.

The sequence of events leading to successful G-seal
10   formation and whole cell recording mode using pre-formed
patch pipettes is as follows:

1.   Selection of a suitable cell.

2.   The patch pipette is positioned approximately 50
microns above the cell.

15   3.   The pipette is lowered until the cell surface is
deformed by the pipette tip.

4.   Negative pressure is applied to the interior of the
pipette until a G-seal is formed between the pipette
tip and the cell membrane.

20   5.   Whole cell recording mode is established by the
application of further negative pressure which
disrupts the cell membrane in the area under the
pipette tip.

Steps two and three are slow and require considerable
25   manual dexterity and a high level of operator skill.
Visualisation of the cells and the patch pipette requires
the use of a high quality microscope and, in order to
position the pipette, a high quality three axis

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micromanipulator with sub-micron resolution in each axis is required.

Summary of the Invention

In its broadest terms the invention provides for one or more cell or cells to be suspended in a liquid medium at a liquid/air interface (by virtue of the effect of surface tension at the interface) whereby the cell or cells are accessible at the interface to a microstructure electrode (such as a pipette tip) to which a cell can attach to form an electrical seal, for the purpose of whole cell voltage clamp recording. According to the invention the electrode can be caused to form a high resistance electrical seal with a cell suspended in the liquid at the liquid/air interface without the need to press the cell against a solid support surface.

Any body of liquid or column of liquid, which gives rise to a situation in which a cell or cells are located in the liquid at a liquid/air interface, can be used in the invention. For examples cells may be suspended in a column of liquid held by surface tension in a capillary tube. Alternatively cells may be suspended in a droplet of liquid, which droplet may itself be suspended from or supported by a support.

It will readily be appreciate that the interface patch clamp technique can be operated in "single cell mode", or could be multiplexed to operate on a matrix of cells with multiple electrodes.

According to one aspect of the invention, interface patching can utilise a patch pipette of conventional type. Cells are supported on a liquid/air interface at one end

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of a capillary tube (e.g. made of glass, polyethylene or
other suitable material). The axis of the patch pipette
is in line with the axis of the tube so that the pipette
tip can be manipulated into the opening of the tube where
5   the cells are supported at the air/liquid interface. The
capillary tube or the patch pipette can be mounted onto a
single axis manipulator. Only one manipulator is required
and this may be used to move either the patch pipette or
the capillary tube. Whole cell recording mode is
10   established as follows:

6.   A layer of cells is established at the interface
       between the extracellular physiological solution (the
       liquid in which the cells are suspended) and air by
       dipping the capillary tube into a suspension of
15   cells. The density of cells in the suspension must
       be sufficient to provide a sufficient number of cells
       to form a layer of cells at the interface.

7.   Electrical contact with the extracellular solution is
       established via a non-polarizable electrode (e.g. an
20   Ag/AgCl wire) and the tube is mounted either to a
       fixed clamp or single axis manipulator.

8.   A patch pipette is provided which can be filled with
       electrolyte solution.

9.   The patch pipette is mounted concentrically with the
25   capillary tube either via a single axis manipulator
       or fixed clamp (if the capillary tube is to be
       moved). The pipette filling solution is connected
       via the non-polarizable electrode to the headstage of
       a conventional patch clamp amplifier. The pipette
30   holder allows suction to be applied to the pipette
       interior.

10. Cell attached patch mode of recording is established
     by bringing the pipette tip in contact with the
     interface by moving the pipette and the capillary
     tube respectively together along the single mounting
     axis (e.g. either by moving the pipette towards the
     tube and interface or vice versa). On entry into the
     interface the movement of the pipette and capillary
     tube together is stopped and the pipette current is
     offset to zero on the patch clamp amplifier. The
     resistance of the pipette increases when the pipette
     contacts one of the cells at the air/liquid
     interface. Suction is then applied to the interior
     of the pipette and the pipette and capillary tube are
     moved closer together until the pipette tip is
     located inside the capillary tube.

     Initial seal formation between the pipette tip and
     the cell may also be assisted by the application of
     gentle suction during entry of the pipette into the
     interface.

     A G-seal is formed between the patch pipette tip and
     the cell membrane by the application of further
     suction to the interior of the pipette and monitoring
     the pipette resistance.

11. Following the formation of cell attached patch mode,
     the suction is released, pipette current is offset to
     zero and a holding voltage applied to the pipette
     (e.g. -60mV).

12. A whole cell recording is obtained by the application
     of further suction to the pipette interior until the
     whole cell recording mode is established in
     conventional manner.

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According to this invention it is preferred that the
capillary tube should be mounted in an upright orientation
(i.e. essentially vertically) with the air/liquid
interface at the downward end of the tube.

5   This has the advantage that suspended cells will tend to
       "sediment" naturally to the downward end of the tube and
       be collected there in a layer. The layer will preferably
       be several cells deep and loosely packed. Thus according
       to the invention the pipette tip may be moved upwardly
10   relative to the air/liquid interface at the tube end
       (either by moving the pipette or the tube along the single
       axis) so as to come into contact with a cell in the layer
       at the interface. The relative density or concentration
       of cells at the interface compared to the density in the
15   bulk of the liquid in the tube ensures a high probability
       that a cell can be collected on the tip without the need
       for visualisation of the operation and without the need
       for multidirectional manipulation of the tip/cell
       positional relationship. Surprisingly it has been found
20   that G-seal formation between the cell and the pipette can
       occur without pressing the cell against a solid substrate.

       Where the arrangement is intended to operate with the
       pipette in an upright orientation (i.e. essentially
       vertically) with the tip uppermost and pointing upwardly,
25   the pipette should be constructed so as to prevent the
       filling electrolyte solution flowing out and being lost.
       This may be achieved for example by use of a custom-made
       mounting assembly and/or by shaping the pipette body to
       prevent loss of filling solution (e.g by bending the
30   pipette shaft into a U- or J- shape).

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The invention also provides methods and apparatus
employing control logic to allow automation of a patch
clamp system employing the Interface Patch Clamp technique
described herein. The logic described will control one or

5   more electromechanical micromanipulators/translators
holding one or more patch clamp pipettes and/or capillary
tubes in order to patch clamp cells and apply
drugs/compounds in order to screen for activity on
membrane ion channels. A major advantage of the logic

10   described is that automation is achieved in this system by
the use of feedback from signals from the patch clamp
amplifier and no image recognition software is required.

The invention is illustrated by way of example in the
accompanying figures in which:

15   Figure 1a shows a capillary tube containing a suspension
of cells; and
Figure 1b shows the cells having formed a layer at the
air/liquid interface at one end of the capillary tube;
Figure 2 shows a general arrangement of the interface

20   patch clamp recording equipment with moveable capillary
tube;
Figure 2a shows an Apparatus for Interface Patch Clamping
with drug/compound application;
Figure 3 shows the cell attached to the patch pipette

25   ready for recording mode.
Figure 4 shows drug/compound addition during interface
patch clamp recording: start position;
Figure 5 shows drug/compound addition during interface
patch clamp recording: extracellular solution added to

30   dish and dish moved down;
Figure 6 shows drug/compound addition during interface
patch clamp recording: solution in dish brought into
contact with interface region;

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Figure 7 shows drug/compound addition during interface
patch clamp recording: capillary raised above surface of
solution in dish;

Figure 8 is a flow diagram of control logic embodying a
further aspect of the invention;

Figure 9 is a flow diagram of an example of the G-seal
formation steps of figure 8;

Figures 10 to 16 are flowcharts of a third embodiment of
the control logic used in the invention, wherein

Figure 10 is a flow diagram of control logic embodying a
further aspect of the invention;

Figure 10a is a flow diagram expanding the reset routine
of figure 10;

Figure 10b is a flow diagram expanding the safe quit
routine of figure 10;

Figure 11 is a flow diagram expanding the interface or
cell locator routine of figure 10;

Figure 11a is a flow diagram expanding the interface hit
routine of figure 11;

Figure 12 is a flow diagram expanding the Giga seal test
routine of figure 10;

Figure 13 is a flow diagram expanding the whole cell
detection routine of figure 10;

Figure 14 is a flow diagram expanding the qualification
routine of figure 10;

Figure 15 is a flow diagram expanding the quality monitor
routine of figure 14;

Figure 16 is a flow diagram expanding the experiment
routine of figure 10;

Figure 16a is a flow diagram expanding the run trace
routine of figure 16;

Figure 16b is a flow diagram expanding the stabilise
routine of figure 16;

Figures 17 to 20 show recordings of current (1) and
voltage (2) obtained from an automated patch clamp system

(AutoPatch) under software control using the Interface
Patch Clamp technique. (Recording from an MK1 cell);
Figure 21 shows the effect of the potassium channel
blocking drug tetraethylammonium (TEA) on the potassium

5   current recorded from MK1 cell in whole cell recording
mode obtained using the Interface Patch Clamp technique;
Figure 22 shows cells suspended in a droplet of liquid
covering a hole through a support surface and providing an
air/liquid interface according to the invention;

10   Figure 23 shows a further embodiment of droplets
containing cell suspensions forming air/liquid interfaces
according to the invention;
Figure 24 shows a multiplexed interface patch clamping
array;

15   Figure 25 shows an alternative form of electrode
microstructure according to the invention; and
Figures 26a and b show a multiwell arrangement for drug
application to patch-clamped cells.

Referring to Figure 1a; a capillary tube (1) of

20   appropriate size can pickup and hold a liquid sample (2)
containing cells (3) in suspension. The sample can be
picked up simply by dipping the tube end into a suitable
bulk liquid reservoir. The liquid in the tube forms an
air/liquid interface (4) at the tube end (5). The cells

25   are initially distributed throughout the liquid relatively
evenly.

Referring to Figure 1b; with the tube in an upright
essentially vertical orientation, the cells tend to
sediment and to pack loosely together at the lower end of

30   the tube by the tube end to form a layer (6) several cells
deep. It will be appreciated by those skilled in the art
that the density and depth of the cell layer can be
determined by such factors as the cell concentration in

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the original suspension, the sedimentation time, the
relative density of the cells and the liquid etc. It will
also be appreciated that means could be devised to
encourage or assist cells to migrate from the liquid
5   towards the air/liquid interface rather than or as well as
relying on gravitational sedimentation alone. The Figure
also shows the top of a patch pipette 8 pointing upwardly
towards the interface.

Referring to Figure 2; an arrangement is shown in which a
10   single axis manipulator is used to move a capillary tube 1
held in a clamp (7) relative to a fixed patch pipette (8)
held in a clamp (9). It will be apparent to those skilled
in the art that this could be reversed so that the pipette
is moved and the tube is fixed. The Figure shows the tube
15   clamped in a linear bearing sliding block (10) attached to
a motorised single axis manipulator (11). The manipulator
should be controlled preferably by computer in order to
allow the motion of the manipulator to be varied by
feedback from the patch clamp amplifier. The patch
20   pipette is provided with a connection (12) to a
conventional headstage. The system is also provided with
a source of variable suction under the control of the
patch clamp amplifier/computer.

In Figure 2a an arrangement is shown in which additional
25   electromechanical micromanipulators have been added. The
micromanipulator labelled (13) is for moving the glass
capillary under automated or manual control. A second
micromanipulator (14) moves the dish for drug application
up and down the glass capillary. A third micromanipulator
30   (15) moves a modified pipette holder to provide electrical
contact with the pipette and a means of applying suction
to the interior of the pipette. Rotational bases (16 and
17) allow the pipette holder to be moved in and out of the

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recording area and rotation of the pipette through 180 degrees for filling with pipette solution.

The Figure also shows additional features, namely; a pipette holder (18); a patch clamp headstage (19); and a
5   dish holder (20).

A version of the apparatus is envisaged in which patch pipettes will be loaded and filled automatically under software control. It is envisaged also that the loading of capillary glass into the apparatus and the filling with
10   cell suspension will also be automated.

Referring to Figure 3; a G-sealed cell 3 is shown held on the tip of the patch pipette 8 and positioned within the entrapped liquid volume in the tube.

Cell attached patch and whole cell (voltage clamp)
15   recording may then be carried out.

The invention described herein has a number of significant features:
   •   Visualisation of the pipette and the cell is not
20   required.
   •   Novel recording configuration that would not be
         considered as obvious.
   •   Surprisingly G-seal formation occurs without pressing
         the cell against a hard substrate.
25   •   Cells form a layer at the solution-air interface.
   •   G-seal formation may be achieved using electronic
         feedback alone.
   •   There is no requirement for optical
         recognition/feedback.
30   •   The system can be automated.

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• Multiple recording capillaries and pipettes may be employed in order to allow recordings to be made simultaneously from many cells.

Exemplary methods of operation of the apparatus of the embodiment under software control to achieve various of these advantages are described below.

In order to use the invention for screening compound (e.g. for ion channel blocking/agonist activity) the compound of interest needs to be applied to the cell attached to the patch pipette. It will readily be appreciated that this could be achieved in different ways, for example by adding the compound to the extracellular liquid in the capillary tube either before or after G-seal formation. One additional advantage of the invention is that the liquid in the tube could be arranged in layers (e.g. containing different compounds or different concentrations of compounds) and the single axis manipulator could then be used to physically move and position a cell on a pipette tip into a chosen layer (e.g. by moving the G-sealed cell on the tip further up the tube away from the air/liquid interface at one tube end).

A further example of how the effects of compounds may be studied is illustrated in figures 4 to 7.

Figure 4 shows a capillary (1) containing the cell suspension (2) and patch pipette (8) in the recording position for whole cell recording from a cell at the pipette tip. In addition, the capillary tube has been inserted through a hole (21) made in a dish (22) (e.g. 35mm plastic culture dish or similar). The dish is made of a material with hydrophobic properties and the hole

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allows the dish to be raised and lowered along the axis of
the capillary by means of a micromanipulator (14).

Figure 5 shows the dish after it has been filled with
extracellular physiological solution (23), which may
contain the drug to be studied, or the drug may be added
at a later stage. Surprisingly, if the fluid level in the
dish is low, leakage through the hole does not occur
because the tendency to leak is counterbalanced by:

1.   The surface tension of the water

2.   The attraction of the water/solution to the glass
      capillary.

After adding the solution to the dish, it is lowered in
the direction of the arrow.

Figure 6 shows the solution in the dish in contact with
the end of the glass capillary and the patch pipette. The
dish and the capillary are now raised simultaneously
(arrows A and B) in order to position the pipette tip/cell
within the layer of liquid in the dish. If drug is
present in the dish at this point and the capillary and
dish were moved upwards rapidly, this would constitute a
rapid application system particularly useful for the study
of agonist responses that desensitise.

Figure 7 shows the effect of raising the capillary so that
it is not in contact with the liquid in the dish. The
pipette tip/cell remains immersed in the external solution
layer in the dish. The solution may be exchanged readily
by perfusion of the dish and this allows multiple drug
additions and dose response curves to be obtained while
recording from the one cell.

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Control Logic for an Automated Patch Clamp System

Introduction

The following describes three embodiments of the control
logic required to allow automation of a patch clamp system
5   employing the Interface Patch Clamp technique described
herein. In each case, the logic described will control
one or more electromechanical
micromanipulators/translators in order to patch clamp
cells and apply drugs/compounds in order to screen for
10   activity on membrane ion channels. A major advantage of
the logic described is that automation is achieved in this
system by the use of feedback from signals from the patch
clamp amplifier and no image recognition software is
required.

15   Methods

*Inputs to the program in all the embodiments are required
from the patch clamp amplifier as follows:*

Imon = current monitor output
Vhold = holding potential

20   *Inputs to the program derived from patch clamp amplifier
output signals are required as follows:*

Inoise = base line current noise recorded from Imon
Rpip = pipette resistance
Rtot = Total resistance
25   Rs derived from Imon signal during voltage step

It is envisaged that these signals and evaluated values
will be obtained from existing software (such as Heka

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software) via a suitable software interface. These
signals and evaluated values are further defined in the
list of variables and parameters below.

Inputs from manipulators/translators are required as
5   *follows, or from the following devices.*

Patch module micromanipulator encoder
Capillary clamp/loader encoder and empty signal
Pipette automated clamp/loader encoder and empty signal
Two axis translator encoders for cell dipper
Drug application micromanipulator encoder
Pipette holder micromanipulator encoder

Control outputs from computer are required for the
following devices.

Patch module manipulator
Pipette automated holder
Capillary loader/clamp
Pipette loader/clamp

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Two axis translator for cell loading system
Pipette clamp
Suction device
Drug application manipulator
Drug perfusion solenoid valve system

The software uses signals derived from the patch clamp
amplifier in order to control a number of peripheral
devices which carry out patch clamping using the Interface
Patch Clamp technique. The devices controlled by the
5   logic comprise a number of micromanipulators, a suction
device for the patch pipette and a valve system for
perfusion of a recording chamber, such as the dish
described above.

A number of parameters are given pre-set values which can
10   be changed by the operator to suit different experimental
conditions.

Summary of the control logic for the automated Interface
Patch Clamp - First Embodiment

Initial seal formation

15   The sequence of movements required for formation of a G-
seal is unique for interface patch clamping and involves
the control of at least one single axis manipulator (e.g.
the patch module motor, although either the pipette or the
capillary may be moved to achieve the necessary relative
20   movement between them) with feedback from the patch clamp
amplifier. In a first embodiment of the control logic,
the pipette is initially spaced from the capillary, as
illustrated in Figure 1b for example, and is moved towards
the liquid/air interface at the capillary end until a
25   change in the current monitor signal is recorded when the
patch pipette enters the liquid/air interface and this

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signal is used as the trigger to stop the
micromanipulator. The pipette resistance may be derived
from the output of the patch clamp amplifier and initial
seal formation is monitored by recording the change in
5   pipette resistance. If the resistance of the pipette does
not increase beyond a pre-set value, the control logic
infers that no G-seal has been formed and activates the
patch module motor to move the liquid/air interface and
the pipette apart until the resistance increases, which
10   may occur when the pipette tip is withdrawn from the
liquid or when a narrow neck of liquid is drawn out by
surface tension between the pipette tip and the capillary
end. When a resistance increase to a pre-set value is
recorded suction is applied to the interior of the patch
15   pipette and the patch pipette and the liquid/air interface
are moved towards each other to a pre-set point, in a
further attempt to form a G-seal with a cell.

Whole cell recording mode

After formation of the cell attached patch clamp recording
20   mode, whole cell mode is obtained by the application of
suction to the interior of the patch pipette while
simultaneously monitoring the current (Imon) for
capacitative transients. In the logic described, the
formation of whole cell recoding mode is detected by a
25   threshold crossing method but it will be apparent to those
skilled in the art that other methods may also be employed
e.g. online FFT (Fast Fourier Transform), Template
Matching etc. The control logic checks for incorrect
detection of whole cell mode before activating the
30   experimental protocol.

Cell quality test

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This routine monitors the quality of voltage clamp by comparing the series recorded resistance with a value related to pipette resistance. It will be appreciated that this method may be further enhanced by relating the acceptable series resistance to the amplitude of current evoked by a voltage step. In addition, an additional loop may be added to include the possibility of recording with a maintained level of suction applied to the pipette in cells that exhibit continuously increasing values of series resistance. The quality of the cell is also monitored by the holding current which should not be more negative than a pre-set value. It will be appreciated that this method could be enhanced by relating the acceptable value for holding current to the amplitude of the current in response to a voltage step.

Drug/compound application

The initial phase of drug application is unique to the interface patch clamp technique and involves the control of two single axis micromanipulators. The movements required utilise the position of the patch module micromanipulator recorded on entry into the interface as a reference point. After the cell has been immersed into solution contained in a perfusion chamber, the control logic calls a routine to carry out perfusion of the chamber via the activation of solenoid flow control valves.

Control Logic in detail   — (First Embodiment)

Variables/parameters

P = Pipette pressure relative to atmospheric pressure defined as 0
d = Patch module motor position
$d0$ = Patch module motor start position
$d1$ = Patch module motor position following entry of pipette into interface
$d2$ = Patch module motor position with pipette in recording position
$d3$ = Patch module motor position for chamber perfusion
$dapp$ = Drug application module micromanipulator position
$dapp0$ = Drug application module micromanipulator position 0
$dapp1$ = drug application micromanipulator position increment 1 Pre-set increment
$dapp2$ = drug application micromanipulator position increment 2 Pre-set increment
Rs = Series resistance
$Cslow$ = Slow capacitance compensation
$Cfast$ = Fast capacitance compensation
$Inoise$ = base line noise
$Rpip$ = pipette resistance
$Rtot$ = Total resistance
$R1$ Intial seal resistance (pre-set value)
$R2$ Seal resistance required for progression to whole cell

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Vhold = Pre set holding potential in mV
Imon = current monitor output
i = pre-set holding current
Ihold base line holding current for holding potential
dpip = pipette holder module motor position
dpip0 = pipette holder module motor position start
dpip1 = pipette holder module motor position pipette on
pclamp = position of pipette clamp/loader encoder
pclamp = 0 pipette not clamped (loading position)
pclamp = 1 pipette clamped (recording position)
rotclamp = position of rotory stage mounting for pipette loader
rotclamp = 0 recording position
rotclamp = 1 pipette filling position
pipfil = pipette filler position
pipfil = 0 pre-/post-fill position
pipfil = 1 fill position
pipsyringe = pipette filler driven syringe position
pipsyringe = dv driven syringe movement required to fill pipette (pre-set value)
cclamp = position of capillary clamp/loader encoder
cclamp = 0 capillary not clamped (loading position)
cclamp = 1 capillary clamped (recording position)
pload = pipette loader empty signal
pload = 0 pipettes in loader
pload = 1 pipette loader empty
cload = capillary loader empty signal
cload = 0 capillaries in loader
cload = 1 capillary loader empty
celldiph = horizontal translator for cell dip
celldiph = 0 cell storage encoder position (pre-set)
celldiph = 1 dip encoder position (pre-set)
celldipv = verticle translator for cell dip
celldipv = 0 pre-/post- dip encoder position (pre-set)
celldipv = 1 capillary dip encoder position (pre-set)
tdelay = variable delay between clamping capillary and starting to patch clamp
dt = time interval
dt1 = pre-set waiting time interval suction off (s)
dt2 = pre-set suction time interval (s)
dt3 = pre-set suction time interval (s)
dt4 = pre-set suction time interval (s)
dt5 = pre-set suction time interval (s)
x = suction increment factor
f = frequency of seal test pulse
detectmin = 0 -ve capacitance transient (3Inoise threshold) not detected
detectmin = 1 -ve capacitance transient (3Inoise threshold) detected
detectmax = 0 +ve capacitance transient (3Inoise threshold) not detected
detectmax = 1 +ve capacitance transient (3Inoise threshold) detected

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I = Whole cell mode flag
I = 0 Not whole cell
I = 1 Whole cell mode established
singlemV = pre-set voltage test pulse
curr = current recorded between pre-set cursors during voltage step
testcurr = pre-set value for current required to start experimental protocol
Valve 1 – 8 = solenoid valves controlling supply of solution to perfusion dish
tv = time interval for valve activation (pre-set)
drain valve = controls suction supply to perfusion dish

Control Logic - Second Embodiment

Control logic according to a further embodiment is
illustrated as a flow diagram in Figure 8. Exemplary
logic steps within each of the functions shown in the flow
5   diagram are set out below.

00 Initialisation

d = d0
dpip = dpip0
pclamp = 0
pipfil = 0
rotclamp = 0
cclamp = 0
celldiph = 0
dapp = dapp0
Rtot >/= 20M
Imon = Inoise
P = 0
If pload = 0 and cload = 0 then GOTO 01
If pload = 1 then report "Re-load pipette cassette" and GOTO 19
If cload = 1 then report "Re-load capillary cassette" and GOTO 19

01 Autofeed

Move capillary clamp motor cclamp = 1
Move celldiph translator to celldiph =1
Move celldipv translator to celldipv =1
Move celldipv translator to celldipv=0
Move celldiph translator to celldiph = 0
start timer
wait for variable delay = tdelay
GOTO 02

02 Pipette load/fill

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```
Move pipette clamp motor pclamp = 1
Move rotation stage 180 degrees rotclamp = 1
move pipfil until pipfil = 1
move motor driven syringe drive until pipsyringe = dv
move pipfil until pipfil = 0
move rotation stage 180 degrees rotclamp = 0
move dpip = dpip1
GOTO 03
```

03 Junction null

```
         Seal test signal on
         Compensate Cfast
         Move patch module down until Imon >/< Inoise
  5      Record patch module motor position d=d1
         Activate Junction null
         Measure Rtot
         Rtot=Rpip
         If Rpip <10M and >/=4M GOTO 04 else Report "Pipette
  10     resistance out of range" and      GOTO 20
```

04 Formation of Gseal

```
         Measure Rtot
         If Rtot=/> 2Rpip
                 Suction on P=-pmmHg
  15             Move patch module down until d=d2
                 (d2 =pre-set recording position)
                 GOTO 05


         04.1
         If Rtot <2 Rpip
  20             Wait for time t1
                 After time t1 move patch module upwards
                 until Rtot>2Rpip then stop.  Wait until
```

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Rtot=2Rpip then move patch module down
until d=d1

If d=d1 and Rtot=/> 2Rpip then suction on
P = -pmmHg
and move patch motor module down until
d=d2, else GOTO 04.1 until maximum of 5
iterations, then GOTO 20

05 Seal test loop

10   05.1 $N=N+1$

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Measure Rtot with -pmmHg for time interval delta t2
If Rtot >/= R1 and dt </= dt2
Then suction off P = P0 until Rtot =/> R2 or dt=t1

If Rtot < R1 and dt >t1
Then repeat 05.1 until N=5 or Rtot =/> R2

If N = 5 and Rtot < R2 Then suction on –xpmmHg
Repeat 05.1 until N = 5 or Rtot =/> R2

If N = 5 and Rtot < R2 Then suction on –xpmmHg
Repeat 05.1 until N = 5 or Rtot =/> R2 or x = pmax

If x=pmax and Rtot < R2 Then Report "Unable to obtain G
seal" GOTO20

If Rtot =/> R2 then GOTO 06 for Whole Cell Mode or
GOTO 08 for Cell Attached Patch Mode

06 Whole Cell - Threshold Method

Compensate Cfast
I = 0
hp = vhold
06.1   Suction on –pmmHg until I=1 or dt= dt3
06.2 Transient detection
N = N+1

If detectmax= 1 and detectmin = 1
Then I = 1
Repeat 06.2 until detectmax = 0 and detectmin = 0 or N=
10

If N = 10 Then GOTO 07

If detectmax = 0 and detectmin = 0
Then GOTO 06.1

07 Cell quality test

Measure Rs and Ihold
If Rs =/> 3Rpip Suction on –pmmHg and start timer

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When Rs < 3Rpip suction off

If Ihold < ipA then and Rs < 3Rpip then seal test signal off
and GOTO 08
If Ihold >ipA and time int = dt4 then GOTO 20

If Rs =/> 3Rpip and time int = dt5 then suction on
–pmmHg

If Rs < 3Rpip and Ihold < ipA then seal test signal off and
GOTO 08

08 Experimental protocol

Apply single voltage step to singlemV
Measure current amp during voltage steps
If curr < testcurr then stop voltage steps
Report "Control current out of range"
GOTO 15

If curr =/> testcurr activate voltage step protocol GOTO 09

Measure Rs
If Rs =/> 3Rpip stop voltage protocol and GOTO 07
Measure Ihold during interval between voltage steps

If Ihold > ipA then stop voltage step/drug application
protocol and GOTO 01

09 Voltage step protocol

Uses program already available
Program must call drug application sub-routine 10

10 Drug/compound application

drain valve on
Fill drug application chamber Valve1 on for time interval

tv

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Move drug application micromanipulator down to d1
—dapp1 (NB —ve value represents downward movement)
Move dapp = d1 + dapp2 and d= d2 + dapp2
simultaneously
Move d = d3 glass capillary moved up
Call software controlling flow control valves

15 Reset Autopatch

GOTO 00.

| 19, 20 | Steps 16 onwards are routines for setting up the apparatus controlled by the software/method and do not relate to the inventive operation of the apparatus and their design is within the normal competency of the skilled person. Steps 19 and 20 relate respectively to reloading the pipette and capillary cassettes and to checking and/or resetting of the apparatus if operation is unsuccessful. |

Figure 9 is a flow diagram illustrating steps 03 and 04,
which relate to formation of the G-seal. These steps
comprise the most important advantageous steps in this
embodiment for controlling the method and apparatus
described herein.

In step 03 (junction null), the pipette tip is initially
spaced below the meniscus at the end of the capillary.
The logic, or software, then controls the patch module
motor to move the pipette tip towards the meniscus until
contact is made, detected by electrical contact
therebetween. The movement is then stopped while the

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pipette resistance is measured and the motor position
recorded (d=d1, as shown in figures 1b and 3). If Rtot is
outside a predetermined range, the experiment is aborted.

In step 04, Rtot is measured and if it is above a
5   predetermined threshold, it is assumed that a cell is
positioned on the pipette tip so suction is applied to the
pipette and the logic controls the patch module motor to
move the pipette tip further into the liquid within the
capillary to a predetermined recording position (d=d2, as
10   shown in figures 1b and 3). The logic then moves to step
05 to test the G-seal.

If at the start of step 04, Rtot is less than the
predetermined threshold, the logic assumes that there is
no cell at the pipette tip. The logic then waits for a
15   predetermined time interval t1 before controlling the
patch module motor to move the capillary away from the
pipette until Rtot is measured to be greater than the
predetermined threshold, when the movement is stopped. It
is believed that in this position the pipette tip is still
20   in contact with the liquid in the capillary but only via a
neck, or bridge, of liquid drawn out by surface tension
between the capillary and the pipette. The logic then
waits until Rtot drops to equal the predetermined
threshold. The logic then controls the patch module motor
25   to return the pipette tip to d=d1, the position when it
first contacted the capillary meniscus in step 03. If
Rtot is then greater than the predetermined threshold it
is assumed that contact with a cell has been made at the
pipette tip, suction is applied to the pipette and the
30   logic controls the patch module motor to move the pipette
into the capillary to the predetermined recording position
at d=d2.

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It is believed that waiting for the time interval t1, which may be between 0.5 and 10 seconds, or preferably about 1 to 5 seconds, permits movement of the cells at the capillary tip, which is encouraged by the movement of the pipette tip to draw out the capillary meniscus.

If Rtot is still less than the predetermined threshold, the steps of waiting for time t1 and slightly moving the pipette are repeated for a predetermined number of iterations until a failure condition (step 20) is reached.

Control Logic - Third Embodiment

Figures 10 to 16 are flow charts illustrating a third embodiment of the control logic of the invention. Aspects of the third embodiment are, where appropriate, common to the first and second embodiments. The third embodiment, however, incorporates certain improvements resulting from experiments by the inventor.

Figure 10 is a flow chart showing all of the operations of the control logic, or software. This is termed the AutoPatch system. Figures 11 to 16, and figures 10a, 10b, 11a, 16a and 16b, are expanded flow charts for operations within the flow chart of Figure 10.

Figure 10 describes the setting up of the AutoPatch system, including the initialisation of all the relevant hardware. This involves the steps up to starting the test sweep 302, after which the steps of interface or cell location 304, Giga seal testing 306, whole cell detection 308, qualification 310 and experiment 312 are performed as described herein. Note that during the patching process, the movements of the capillary and the petri dish are locked together by the software in order to maintain their

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positions relative to each other constant. The movement
of the petri dish has no role in the patching process.

Initially the capillary and petri dish move towards the
pipette at a rapid speed to a pre-set distant (step 314)
5   in order to position the liquid/air interface within
approximately 1mm of the pipette tip. This initial
(rough) position is performed in order to shorten the time
interval between starting the patching process and
reaching the interface. The distance between the patch
10   pipette tip and the liquid/air interface is initially
larger than 1mm in order to allow sufficient space for
loading of the pipette and the capillary. After rough
positioning, the seal test pulse is started (step 316) and
the translators (capillary and petri dish) are switched to
15   slow speed prior to entry of the pipette into the
liquid/air interface.

Figures 10a and 10b expand the safe quit routine 318 and
the reset routine 320 used at various points by the
control logic or software.

20   Figure 11 is a flow diagram expanding the interface or
cell locator routine 304 of Figure 10. In this routine,
suction is applied to the interior of the pipette, and the
capillary and petri dish are moved towards the pipette at
a slow rate (e.g. 10 micrometers/s) (step 322). The
25   current is monitored after each sweep to determine when
the interface is detected (step 324). The interface is
detected by an offset in the baseline current or the
appearance of a current pulse when the circuit is made
326. In contrast with conventional patch clamp, seal
30   formation with the Interface Patch Clamp technique can
occur virtually simultaneously with contact between the
pipette tip and the external bathing solution at the

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interface 328. The logic distinguishes between an open circuit (pipette tip in air) and a rapidly formed seal by monitoring the current trace for the appearance of a capacitance transient at the end of the seal test pulse

5   (step 326). This transient is due to the pipette capacitance, which increases as the pipette is immersed into the solution at the interface. As the pipette tip touches or crosses the air/liquid interface the capacitance is likely to be undetectable amongst

10   electrical noise. As the pipette tip moves into the liquid, however, the capacitance increases until it is detectable over the noise.

After the pipette has entered the interface, the capillary (and movement of the petri dish) is stopped, a junction

15   null may be performed and the pipette resistance is monitored. The presence of a cell on the pipette tip is indicated by an increase in pipette resistance which must reach a pre-set value before the logic will allow the system to proceed to the next step. After a cell has been

20   detected the Giga seal test 306 is undertake.

Figure 11a expands the interface hit routine 330 of the interface or cell location routine 304 of Figure 11.

Figure 12 is a flow diagram of the Giga seal test routine 305 carried out after contact with a cell has been

25   detected in the interface or cell location routine 304. The Giga seal test routine 306 comprises a repetitive loop 332 in which the level of suction applied to the pipette interior is increased in pre-set increments 334 and times 336 while monitoring the change in pipette resistance.

30   The suction is increased until the maximum vacuum is obtained or Giga seal formation occurs. The loop ends and the suction is switched off 338 if either of these

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conditions is satisfied. If the maximum suction has been
applied but a Giga seal has not formed the loop is
repeated until Giga seal formation or time-out. The
formation of a Giga ohm seal is required in order to allow
5   progression to the next stage.

Figure 13 expands the whole cell detection routine 308 of
Figure 10. In this routine, the transients due to the
pipette capacitance are cancelled and this is followed by
a repetitive loop 340 in which the level of suction
10   applied to the pipette interior is increased in pre-set
increments 342 and times 344 while monitoring the current
346 for the appearance of capacitance transients. These
transients are due to the charge and discharge of the cell
membrane capacitance and are indicative of the formation
15   of whole cell recording mode.

Figure 14 expands the qualification routine 310 of Figure
10. In order to qualify for use in an experiment the cell
must exhibit a voltage gated (or other) current equal to
or greater than a pre-determined amplitude and polarity
20   348 in response to a test pulse. Qualification proceeds
until the cell qualifies or timeout is hit. During
qualification the quality monitor 350 is run also.

Figure 15 is a flow diagram of the quality monitor routine
350 of Figure 14 and 16, described below. It comprises a
25   repetitive loop in which the pipette suction is varied in
response to measurements of series resistance (RSeries)
and current (IMon). Current flow through the cell
membrane via the pipette generates a voltage error due to
RSeries. The value of RSeries often increases during
30   whole cell recording and this effect can be reduced by the
application of suction to the pipette interior 352. An
increase in the value of the current at the holding

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potential (usually - 60mV) indicates loss of the Giga seal
and this can be caused by excessive suction. Acceptable
values for RSeries and IMon are entered in the settings
for the software. The quality monitor runs both during
5   the qualification stage and the experiment.

Figure 16 expands the experiment routine 312 of Figure 10.
In the experiment routine, movements of the petri dish and
capillary required to carry out drug application by the
method shown in figures 4-7 inclusive are carried out.
10   During these movements the dish is filled with external
solution/external solution plus drug via solenoid operated
flow control values 358. Before drug is added, the
current evoked by the test pulse (or pulses) must be
reproducible within a pre-determined percentage (entered
15   in the settings).

Figures 16a and 16b expand the trace run routine 354 and
the stabilise routine 356 of the experiment routine 312.

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Figure 17 shows recordings of current (1) and voltage (2)
obtained from an automated patch clamp system (AutoPatch)
showing the formation of a Giga Seal under software
control using the Interface Patch Clamp technique.

5   Recordings from an MK1 cell.

Figure 18 shows recordings of current (1) and voltage (2)
from an automated interface patch clamp system (AutoPatch)
showing the increase in capacitance transient observed
after moving the capillary to position d2 (records a and

10   b). (c) and (d) were obtained after automatic
compensation for pipette capacitance and a change in the
holding potential to -60mV. Recording obtained from the
same cell as Figure 17.

Figure 19 shows recordings of current (1) and voltage (2)

15   from an automated interface patch clamp system
(AutoPatch). Records (a) and (b) were obtained after Giga
seal formation (cell attached patch mode) using the
interface patch clamp technique. The application of
suction to the pipette interior under full software

20   control ruptured the membrane patch to obtain the whole
cell recordings shown in (c) and (d). The establishment
of the whole cell mode of recording is shown by the
presence of the large capacitance transients on the
current trace. Recording obtained from the same cell as

25   figures 17 and 18.

Figures 20 shows recordings of membrane current (1) and
voltage (2) in whole cell recording mode obtained using an
automated patch clamp system (AutoPatch) which employs the
interface patch clamp technique. The holding potential

30   was -60mV and (a) and (b) show outward potassium currents
(Kv1.1) in response to a voltage step to +30mV. Recording
obtained from the same cell as figures 17, 18 and 19.

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Figure 21 shows the effect of the potassium channel
blocking drug tetraethylammonium (TEA) on the potassium
current recorded from MK1 cell in whole cell recording
mode obtained using the Interface Patch Clamp technique.

5   After the establishment of whole cell recording mode the
cell was positioned in a recording dish by the method
shown in figures 4 to 7 and described in the text.
(a) shows the current obtained in normal extracellular
solution

10   (b) shows the effect of replacing the solution in the dish
with extracellular solution containing TEA (5mM).
(c) the blocking effect of TEA was reversed by washing.

It will be readily appreciated by those skilled in the art
that:

15   1.   The stability of recording using the interface patch
clamp technique may be superior to that of
conventional patch clamping. The greater stability
of interface patch clamping is because the cell is
held by the patch pipette alone. In conventional

20   patch clamp recordings the cell is held by the path
pipette and a solid substrate and vibration tends to
move the pipette relative to the substrate causing
loss of the G-seal. The interface patch clamp is, in
contrast to conventional patch clamp apparatus,

25   relatively insensitive to vibration during drug
application.

2.   This method of drug application could be applied to a
plurality of recording pipettes/capillaries and form
the basis for a high throughput electrophysiological

30   assay system. It will readily be appreciated that
the Interface Patch Clamp technique could be used
with multiple pipettes and multiple capillaries in a

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manner in which each pipette enters its respective
aligned capillary either individually in sequence or
all together. Although not currently preferred, a
single pipette could be used which is caused to enter
5   more than one capillary sequentially. Multiple patch
clamp recordings could be made either sequentially or
simultaneously, depending on the application.

As was mentioned above, it is not essential to the general
10   principle of the invention to use a capillary in order to
create a column of liquid which gives rise to a liquid/air
interface at which cells can be located. Other ways can
be envisaged in which the same effect can be achieved.
For example, as shown in figure 22, a droplet or "blob" of
15   liquid may be provided on a support surface. The surface
has a hole through it and the droplet covers the hole.
Surface tension prevents the liquid from the droplet
dropping through the hole. Within the droplet cells are
suspended. This allows access to the droplet and the
20   cells contained therein by a suitable electrode such as a
patch pipette. In the arrangement shown in figure 22,
means are provided for flow of other liquids in to and out
of a dish or other container of which the support surface
with the hole in it forms a wall. Once a cell has been
attached to the electrode, other liquids may be introduced
25   into the container either in batch mode or in flow-through
mode in order to result in the cell being exposed at its
external surface to the surrounding liquid. Clearly in
such an arrangement, the original liquid and the remaining
un-attached cells will tend to be washed away from the
30   area of the electrode/pipette.

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It is within the scope of the invention that droplets
might be provided on non-perforate support surfaces. As
shown in figure 23, the effect of surface tension may be
to allow droplets of a suitable liquid to adhere
5   automatically to the underside of a suitable support
surface. The support surface might for example be a cover
slip of glass or other material. Droplets in which cells
are suspended provide the air/liquid interface according
to the invention and consequently may be used in a method
10   of interface patch clamping as described above.

As has already been mentioned, the arrangement shown in
Figure 23 as well as the arrangement shown in Figure 24
allows for the formation of a matrix of cell suspensions
so that multiple electrodes can be multiplexed to take
15   readings either simultaneously or sequentially (as well as
singly).

It will be appreciated by those skilled in the art that a
conventional glass "patch pipette" could be replaced by an
equivalent electrode. It is considered to be within the
20   scope of the present invention that the electrode might be
either a single region or a matrix of regions on a sheet
of material (such as a silicon wafer) which incorporates a
microstructure to which a cell can be attached and which
would provide the necessary electrical connection. For
25   example, as shown in Figure 25, microstructures could be
etched on to a silicon wafer (e.g. an oxidised silicon
wafer), which microstructures would be designed and
adapted to be able to capture a cell from the liquid/air
interface of an arrangement according to the present
30   invention. Thus, the performance and advantage of the
invention is not limited to the currently preferred

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conventional glass patch pipette but would include
functionally equivalent means.

As has been described before, a drug in liquid solution
can be applied to the cell in a number of ways. For
example the drug could be applied via the capillary if the
air interface is formed in a capillary tube.
Alternatively the drug can be applied by perfusion into a
dish (as described with reference to figures 4 to 7).
Furthermore, perfusion could be achieved by flowing the
drug-containing liquid through a dish or container as
shown in Figure 22.

A further arrangement for drug application is shown in
Figure 26a and 26b. In this case the electrode (for
example the patch pipette) penetrates through the lower
wall of a well. A suspension of cells is loaded in to a
capillary tube as previously described. Attachment of a
single cell to each pipette tip follows, as described
before. Once cells are attached to the pipette tips the
capillary tubes containing the remainder of the cells in
suspension can be removed. Subsequently, a drug solution
(23) is dispensed into each well (Figure 26b) and patch
clamp measurements can then be carried out on the cell in
the environment of the surrounding drug solution.

Optimisation of Patch Clamping Conditions

Those skilled in the art will appreciate that within the
general teaching contained herein for the interface patch
clamping method and apparatus, it may be necessary to
optimise certain conditions for patch clamp measurements.
For example the concentration and packing density of cells
in the suspension may need to be optimised. Furthermore,
the cells and/or solutions may be temperature sensitive

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Claims

1.   A method for providing a cell attached to a patch clamp electrode and having a high resistance (Giga Ohm) electrical seal between an area of the cell membrane and the electrode, which includes the steps of:

i) providing a suspension of cells in a liquid;

ii) causing the formation of a layer of cells at the interface between the air and the liquid in which the cells are suspended;

iii) bringing the patch clamp electrode into contact with the interface by moving one or both of the electrode and the interface respectively together;

iv) contacting the electrode with a cell in the cell layer at or near the interface; and

v) causing attachment of the cell to the electrode.

2.   A method according to claim 1 for providing a cell attached to the tip of a patch clamp pipette and having a high resistance (Giga Ohm) electrical seal between an area of the cell membrane and the tip; wherein the electrode of claim 1 is the tip of a patch clamp pipette; which method includes the steps of:

i) providing a capillary tube containing a suspension of cells in a liquid;

ii) causing the formation of a layer of cells at one end of the capillary tube at the interface between the air and the liquid in which the cells are suspended;

iii) bringing the tip of the patch clamp pipette into contact with the interface by moving one or both of the pipette and the tube respectively together along a common axis of movement;

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AMENDED SHEET